

**CLAIMS**

What is claimed is:

- 5 1. A truncated glial cell line-derived neurotrophic factor (GDNF) protein product having an amino acid sequence

X-[Cys<sup>41</sup>-Cys<sup>133</sup>]-Y

wherein

[Cys<sup>41</sup>-Cys<sup>133</sup>] represents the amino acid sequence of Cys<sup>41</sup> through Cys<sup>133</sup> as depicted in Figure 1 (SEQ ID NO 2);

Y represents the carboxy terminal group of Cys<sup>133</sup> or a carboxy-terminus amino acid residue of Ile<sup>134</sup>; and

X represents a methionylated or nonmethionylated amine group of Cys<sup>41</sup> or amino-terminus amino acid residue(s) selected from the group:

15

|  |  |
|--|--|
| G                                      |  |
| RG                                     |  |
| NRG                                    |  |
| KNRG (SEQ ID NO:3)                     |  |
| GKNRG (SEQ ID NO:4)                    |  |
| RGKNRG (SEQ ID NO:5)                   |  |
| QRGKNRG (SEQ ID NO:6)                  |  |
| GQRGKNRG (SEQ ID NO:7)                 |  |
| RGQRGKNRG (SEQ ID NO:8)                |  |
| RRGQRGKNRG (SEQ ID NO:9)               |  |
| G RRGQRGKNRG (SEQ ID NO:10)            |  |
| KG RRGQRGKNRG (SEQ ID NO:11)           |  |
| GKG RRGQRGKNRG (SEQ ID NO:12)          |  |
| RGKG RRGQRGKNRG (SEQ ID NO:13)         |  |
| SRGKG RRGQRGKNRG (SEQ ID NO:14)        |  |
| NSRGKG RRGQRGKNRG (SEQ ID NO:15)       |  |
| ENSRGKG RRGQRGKNRG (SEQ ID NO:16)      |  |
| PENSRGKG RRGQRGKNRG (SEQ ID NO:17)     |  |
| NPENSRGKG RRGQRGKNRG (SEQ ID NO:18)    |  |
| ANPENSRGKG RRGQRGKNRG (SEQ ID NO:19)   |  |
| A ANPENSRGKG RRGQRGKNRG (SEQ ID NO:20) |  |

|           |            |            |                               |
|-----------|------------|------------|-------------------------------|
|           | AA         | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:21)     |
|           | AAA        | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:22)     |
|           | QAAA       | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:23)     |
|           | RQAAA      | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:24)     |
|           | NRQAAA     | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:25)     |
|           | RNRQAAA    | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:26)     |
|           | ERNRQAAA   | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:27)     |
|           | RERNRQAAA  | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:28)     |
|           | RRERNRQAAA | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:29)     |
| P         | RRERNRQAAA | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:30)     |
| LP        | RRERNRQAAA | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:31)     |
| VLP       | RRERNRQAAA | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:32)     |
| AVLP      | RRERNRQAAA | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:33)     |
| MAVLP     | RRERNRQAAA | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:34)     |
| QMAVLP    | RRERNRQAAA | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:35)     |
| KQMAVLP   | RRERNRQAAA | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:36)     |
| DKQMAVLP  | RRERNRQAAA | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:37) and |
| PDKQMAVLP | RRERNRQAAA | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:38)     |

and addition, substitution and internal deletion variants and derivatives thereof.

2. A truncated GDNF protein product according to Claim 1, wherein X =  
5 RQAAA ANPENSRGKG RRGQRGKNRG (SEQ ID NO:24) or a variant thereof.
3. A truncated GDNF protein product according to Claim 1, wherein X =  
NPENSRGKG RRGQRGKNRG (SEQ ID NO:18) or a variant thereof.
- 10 4. A truncated GDNF protein product according to Claim 1, wherein X =  
PENSRGKG RRGQRGKNRG (SEQ ID NO:17) or a variant thereof.
5. A truncated GDNF protein product according to Claim 1, wherein X =  
SRGKG RRGQRGKNRG (SEQ ID NO:14) or a variant thereof.  
15
6. A truncated GDNF protein product according to Claim 1, wherein X =  
RGQRGKNRG (SEQ ID NO:8) or a variant thereof.

7. A truncated GDNF protein product according to Claim 1, wherein X = GQRGKNRG (SEQ ID NO:7) or a variant thereof.
8. A truncated GDNF protein product according to Claim 1, wherein X =  
5 KNRG (SEQ ID NO:3) or a variant thereof.
9. A truncated GDNF protein product according to Claim 1, wherein X = NRG  
or a variant thereof.
- 10 10. A truncated GDNF protein product according to Claims 1 through 9,  
wherein said amino acid sequence is glycosylated.
11. A truncated GDNF protein product according to Claims 1 through 9,  
wherein said amino acid sequence is nonglycosylated.
- 15 12. A truncated GDNF protein product according to Claim 1, wherein said  
derivative is an X-[Cys<sup>41</sup>-Cys<sup>133</sup>]-Y amino acid sequence conjugated to a water  
soluble polymer.
- 20 13. A polynucleotide encoding a truncated GDNF protein according to Claim 1.
14. A polynucleotide according to Claim 13, comprising a portion of the  
sequence as set forth in Figure 1.
- 25 15. A polynucleotide according to Claim 13, comprising a portion of the  
sequence as set forth in Figure 3.
16. A polynucleotide according to Claim 13, comprising a portion of the  
sequence as set forth in Figure 4.
- 30 17. A polynucleotide according to Claim 13, comprising the sequence as set  
forth in Figure 5.
18. A polynucleotide according to Claim 13, comprising the sequence as set  
35 forth in Figure 6.

19. A polynucleotide according to Claim 13, comprising the sequence as set forth in Figure 7.
20. A vector, comprising a polynucleotide of Claim 13 operatively linked to  
5 an expression control sequence.
21. A prokaryotic or eukaryotic host cell transformed or transfected with a polynucleotide of Claim 13.
- 10 22. A method for producing a truncated GDNF protein, comprising growing host cells of Claim 21 in a suitable nutrient medium and, optionally, isolating said truncated GDNF from said cells or said nutrient medium.
- 15 23. A method for producing a truncated GDNF protein according to Claim 22, wherein said host cells are *E. coli*.
24. A method for producing a truncated GDNF protein according to Claim 22, wherein said host cells are Chinese hamster ovary cells.
- 20 25. A method for the production of a truncated glial cell line-derived neurotrophic factor (GDNF) protein, comprising the steps of:  
(a) culturing a prokaryotic or eukaryotic host cell transformed or transfected with a vector of Claim 20;  
(b) maintaining said host cell under conditions allowing the expression of  
25 truncated GDNF protein by said host cell; and  
(c) optionally isolating the truncated GDNF protein expressed by said host cell.
- 30 26. A truncated GDNF protein which is the recombinant expression product of a prokaryotic or eukaryotic host cell containing an exogenous polynucleotide of Claim 13.
- 35 27. A pharmaceutical composition comprising a truncated GDNF protein product according to Claim 1 in association with a pharmaceutically acceptable vehicle.

28. A pharmaceutical composition comprising a truncated GDNF protein, produced in accordance with the method of Claim 22 in association with a pharmaceutically acceptable vehicle.
- 5 29. A pharmaceutical composition comprising a truncated GDNF protein, produced in accordance with the method of Claim 25 in association with a pharmaceutically acceptable vehicle
30. A method of treating Parkinson's Disease comprising administering to a  
10 patient the pharmaceutical composition of Claim 27.
- 15 31. A method of treating Parkinson's Disease comprising administering to a patient a polynucleotide sequence of Claim 13 to provide *in vivo* production of said truncated GDNF protein.
32. A method of treating Parkinson's Disease comprising implanting in a patient a cell transformed with a polynucleotide sequence of Claim 13 to provide *in vivo* production of said truncated GDNF protein.
- 20 33. A glial cell line-derived neurotrophic factor (GDNF) composition, comprising a mature GDNF protein and one or more truncated GDNF proteins, wherein said mature GDNF protein has a molecular weight of approximately 44 kDa, and wherein said truncated GDNF protein(s) has a molecular weight of approximately 29 to 40 kDa.
- 25 34. A GDNF composition according to Claim 33, comprising at least two species of truncated GDNF protein wherein a first species has a molecular weight of approximately 36 kDa and a second species has a molecular weight of approximately 40 KDa.
- 30 35. A GDNF composition according to Claim 34, wherein said second truncated GDNF species having a molecular weight of approximately 40 kDa is a heterodimer of a mature GDNF monomer having a molecular weight of approximately 22 kDa and a truncated GDNF monomer having a molecular weight of approximately 18 kDa.

36. A truncated GDNF protein isolated from the GDNF composition of Claim 33 and having a molecular weight of approximately 29 to 40 kDa.

37. A truncated GDNF protein isolated from the GDNF composition of 5 Claim 33 and having a molecular weight of approximately 29 to 36 kDa.

38. A truncated GDNF protein derived from a mature GDNF protein expressed by a recombinantly modified bacterial or mammalian cell, said truncated GDNF protein having an amino acid sequence

10 X-[Cys<sup>41</sup>-Cys<sup>133</sup>]-Y

wherein

[Cys<sup>41</sup>-Cys<sup>133</sup>] represents the amino acid sequence of Cys<sup>41</sup> through Cys<sup>133</sup> as depicted in Figure 1 (SEQ ID NO 2);

15 Y represents the carboxy terminal group of Cys<sup>133</sup> or a carboxy-terminus amino acid residue of Ile<sup>134</sup>; and

X represents an amine group of Cys<sup>41</sup> or amino-terminus amino acid residue(s) selected from the group:

|                                    |  |
|------------------------------------|--|
| G                                  |  |
| RG                                 |  |
| NRG                                |  |
| KNRG (SEQ ID NO:3)                 |  |
| GKNRG (SEQ ID NO:4)                |  |
| RGKNRG (SEQ ID NO:5)               |  |
| QRGKNRG (SEQ ID NO:6)              |  |
| GQRGKNRG (SEQ ID NO:7)             |  |
| RGQRGKNRG (SEQ ID NO:8)            |  |
| RRGQRGKNRG (SEQ ID NO:9)           |  |
| G RRGQRGKNRG (SEQ ID NO:10)        |  |
| KG RRGQRGKNRG (SEQ ID NO:11)       |  |
| GKG RRGQRGKNRG (SEQ ID NO:12)      |  |
| RGKG RRGQRGKNRG (SEQ ID NO:13)     |  |
| SRGKG RRGQRGKNRG (SEQ ID NO:14)    |  |
| NSRGKG RRGQRGKNRG (SEQ ID NO:15)   |  |
| ENSRGKG RRGQRGKNRG (SEQ ID NO:16)  |  |
| PENSRGKG RRGQRGKNRG (SEQ ID NO:17) |  |

|            |            |  |
|------------|------------|--|
|            | NPENSRGKG  | RRGQRGKNRG (SEQ ID NO:18)                |
|            | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:19)                |
| A          | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:20)                |
| AA         | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:21)                |
| AAA        | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:22)                |
| QAAA       | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:23)                |
| RQAAA      | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:24)                |
| NRQAAA     | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:25)                |
| RNRQAAA    | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:26)                |
| ERNRQAAA   | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:27)                |
| RERNRQAAA  | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:28)                |
| RRERNRQAAA | ANPENSRGKG | RRGQRGKNRG (SEQ ID NO:29)                |
| P          | RRERNRQAAA | ANPENSRGKG RRGQRGKNRG (SEQ ID NO:30)     |
| LP         | RRERNRQAAA | ANPENSRGKG RRGQRGKNRG (SEQ ID NO:31)     |
| VLP        | RRERNRQAAA | ANPENSRGKG RRGQRGKNRG (SEQ ID NO:32)     |
| AVLP       | RRERNRQAAA | ANPENSRGKG RRGQRGKNRG (SEQ ID NO:33)     |
| MAVLP      | RRERNRQAAA | ANPENSRGKG RRGQRGKNRG (SEQ ID NO:34)     |
| QMAVLP     | RRERNRQAAA | ANPENSRGKG RRGQRGKNRG (SEQ ID NO:35)     |
| KQMAVLP    | RRERNRQAAA | ANPENSRGKG RRGQRGKNRG (SEQ ID NO:36)     |
| DKQMAVLP   | RRERNRQAAA | ANPENSRGKG RRGQRGKNRG (SEQ ID NO:37) and |
| PDKQMAVLP  | RRERNRQAAA | ANPENSRGKG RRGQRGKNRG (SEQ ID NO:38)     |

and addition, substitution and internal deletion variants thereof.

39. A truncated GDNF protein according to Claim 38, wherein X is selected  
5 from the group consisting of:

G  
RG  
NRG  
KNRG (SEQ ID NO:3)  
GKNRG (SEQ ID NO:4)  
RGKNRG (SEQ ID NO:5)  
QRGKNRG (SEQ ID NO:6)  
GQRGKNRG (SEQ ID NO:7)  
RGQRGKNRG (SEQ ID NO:8) and  
RRGQRGKNRG (SEQ ID NO:9)

and variants thereof.

40. A truncated GDNF protein according to Claim 38, wherein mature GDNF protein is expressed by a recombinantly modified bacterial cell and said  
5 truncated GDNF protein is produced *in vitro* or *in vivo*.

41. A method of preparing a pharmaceutical composition wherein a therapeutically effective amount of a truncated GDNF protein product according to Claim 1 is mixed with one or more pharmaceutically acceptable vehicles.

10 42. The use of a truncated GDNF protein product according to Claim 1 for treating damage to the nervous system caused by disease or injury.

15 43. The use of a truncated GDNF protein product according to Claim 42 for treating Parkinson's disease.

44. The use of a truncated GDNF protein product according to Claim 1 for the preparation of a pharmaceutical composition for treating damage to the nervous system caused by disease or injury.

20

Add A3